

EVALUATION OF SALINITY STRESS ON EARLY PERFORMANCE OF COWPEA (*VIGNA UNGUICULATA* L. Walp)

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Abstract

Cowpea (*Vigna unguiculata* L. Walp) is the second most important legume after bean in Kenya. Cowpea grains and leaves are mainly used for human and animal consumption. In Kenya, low production of cowpea among small scale farmers is attributed to both biotic (pests and diseases) and abiotic factors (salinity). Salinity inhibits metabolic processes and constrains plant growth which impacts on crop productivity in the majority of agricultural fields. Cowpea is mostly grown on arid and semi-arid regions which are prone to salinity. The objective of this study was to assess the germination in cowpea subjected to sodium chloride and calcium chloride which are major ions in saline soils. Four cowpea varieties (K80, M66, Kunde 1 and KVU 27-1) that are commonly grown in Kenya were studied. Seeds were sown in inert sand in a greenhouse. The electrical conductivity of both CaCl_{2(aq)} and NaCl_(aq) were adjusted to four treatments of each salt: 0 for control, 4, 8 and 12 dS/m in replicates of three. The experiment design was 4 x 8 factorial experiment laid out in complete randomized design. Data on number of seeds which germinated, germination associated parameters and days to emergence was obtained. Data was subjected to one way ANOVA means separated by LSD $\alpha = 0.05$. Results showed that as each salt level increased, days to emergence increased and the number of seeds which germinated, final germination percentage and seedling vigour declined. M66 recorded the least days to emergence, highest number of seeds, final germination percentage and seedling vigour followed by KVU 27-1, K80 and Kunde 1 respectively. It was concluded that M66 was the most tolerant variety while Kunde 1 was significantly affected by salinity levels above 8 dS/m. Farmers in saline affected areas should adopt growing M66 cowpea variety.

Keywords: Cowpeas, Germination, Salinity, Tolerance, Salt Stress, Adaptation

Introduction

Cowpea (*Vigna unguiculata* (L). Walp) is an annual crop grown for its edible seeds, immature pods and leaves. The seeds are high in protein (23 – 32 %). Additionally, the leaves are rich in proteins, minerals and the whole plant is used as forage for livestock. Due to its tolerance to water stress, it's an important crop in Sub-Saharan Africa and also in tropical and subtropical regions. The World production of the crop is estimated at over 8.9 million MT per year on about 14.4 million hectares. Over 95% of the global production is in Africa, with

Nigeria being the world's largest producer (FAO *et al.*, 2020).

Large areas of land have potential for cultivation in arid and semi-arid regions provided that they can be irrigated. In spite of this, about 23 % of the world's cultivated lands are saline and about 37 % are sodic (Khan *et al.*, 2011). Kenya is 80 % semi-arid and arid, and according to Mugai (2004), 40 % of soils in these regions are saline. Salinity in soils is mainly caused by natural processes such as mineral weathering and anthropogenic activities such as irrigation. The ions

responsible for salinity are Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , CO_3^{2-} and SO_4^{2-} .

According to Haque, (2006), salinity reduces up to 50 % of crop yields by affecting germination, growth, quality and quantity of plant biomass through osmotic stress and ion toxicity. High salinity levels lead to reduction in and delayed germination in both halophyte and glycophyte seeds (Gul *et al.*, 2013). Physical processes of water uptake resulting in seed hydration promote activation of metabolic processes thus breaking seed dormancy. High salinity levels inhibit water uptake by seeds due to high osmotic potential hence preventing their germination. Sodium chloride in addition may be inhibitory to the activities of some enzymes that play critical roles in breaking dormancy in seed (Sanchez *et al.*, 2014).

Salinity affects germination of seeds through reduction in the mobilization of reserve foods, delaying cell division, enlarging and injuring hypocotyls (Rahman *et al.*, 2013). Other physiological responses to salinity include reduction of reserve foods, delaying cell division, enlarging and injuring hypocotyls (Rahman *et al.*, 2013). Seedling growth is critical in the life cycle of plant growth and development (Llanes *et al.*, 2016). Under salt stress, elongation and rate of growth of coleoptiles decreases due to low soil water potential, and seedlings may not get established properly due to the weak coleoptiles and root growth. Reduced seedling growth in barley has been reported by Cokkizgin, (2012) and on *Phaseolus* by Bayuelo-Jiménez *et al.* (2002). Adaptation of plants to salinity during germination and early development of the seedling is necessary for the establishment of species in stressful localities. Cabrera (2000) reported that greater tolerance to salinity during germination is associated with lower respiration rates and greater reserve of respiratory substances.

Soil salinity can be reduced by application of excess irrigation water which leaches out the soluble salts which may also alter the soil fertility (Li *et al.*, 2014). Given the increasing scarcity and competition for water resources, increasing drainage for better flushing of salts on soil surface is not a sustainable option to alleviate salinity problems in arid and semi-arid areas. In order to mitigate salinity effects on crop productivity, the most viable option is use of salt-tolerant crops, which is a promising approach that may satisfy the increased food demand in the world (Higbie *et al.*, 2010). Therefore, the study evaluated germination of cowpeas (*Vigna unguiculata* L. Walp) varieties subjected to different saline conditions to establish their salinity tolerance capacity. Therefore, the study evaluated germination of cowpeas (*Vigna unguiculata* L. Walp) varieties subjected to different saline conditions to establish their salinity tolerance capacity.

MATERIALS AND METHODS

Study area

This study was carried out in a greenhouse at Jomo Kenyatta University Science and Technology (1°10'50.0"S, 36°55'41.0"E), Juja Sub-county located in Kiambu County. The greenhouse whose mean minimum and maximum air temperatures during the growth period were 16 and 32 °C, respectively.

Planting materials

Four varieties of grain cowpea (*Vigna unguiculata* L. Walp); K80, Kunde 1, M66 and KVU 27-1 were obtained from the Kenya Agricultural & Livestock Research Organization station in Machakos. Seed viability was determined by floatation in water; the ones that floated were discarded and those that sunk were deemed potentially viable (ISTA, 2009).

Experimental design

Four varieties; K80, Kunde 1, M66 and KVU 27-1 were evaluated under four salinity

concentrations; 0 (control), 4, 8 and 12 dS/m each for $\text{CaCl}_{2(\text{aq})}$ and $\text{NaCl}_{(\text{aq})}$. The treatments were each replicated three times. The experiment design was 4 x 8 factorial experiment laid out in complete randomized design. Salinity levels were maintained throughout the experiment by use of an Electrical Conductivity probe (Malarde *et al.*, 2008).

Crop performance and data collection

Ten seeds from each of the four varieties of cowpeas were planted in plastic punnet pots measuring 15 * 10 cm in replicates of three. The punnets were $\frac{3}{4}$ filled with inert sand, and the seeds were planted at a depth of 5 cm. The days to emergence, height of seedling, and number of seedlings emerging was scored daily and were used to calculate the germination variables; mean germination time, mean germination rate, final germination percentage and seedling vigour (Ferreira *et al.*, 2009).

One way Analysis of variance was used to determine the variation of the varieties for germination parameters under the different levels of salts applied. Comparisons of the means were done using Fisher's protected Least Significant Difference (LSD) test at 5% confidence interval. Statistical analyses were performed using SPSS (software version 21).

RESULTS

Effect of NaCl and CaCl_2 levels on days to emergence

The number of days to emergence of cowpea varieties treated with different levels of $\text{NaCl}_{(\text{aq})}$ and $\text{CaCl}_{2(\text{aq})}$ was analysed for variation. The higher the level of salts, the more the days the four cowpea varieties took

to germinate. Under $\text{NaCl}_{(\text{aq})}$ means of days to emergence were only significantly different in K80. Although M66 recorded the fewest days to emergence (7) while Kunde 1 recorded the highest (10) as shown in Table 1 but the means were not different within the two varieties. Under $\text{CaCl}_{2(\text{aq})}$ differences in days to emergence were not significant at $p \leq 0.05$ in all varieties as salinity levels increased as shown in Table 2.

Effect of NaCl and CaCl_2 levels on cowpea seeds germination

The highest number of seeds which germinated was in 0 dS/m while 12 dS/m had the lowest. Under NaCl levels, the means of seeds which germinated were significantly different in M66, Kunde 1 and KVU 27-1. M66 recorded the highest number of seeds (7.3) which germinated at 12 ds/m while Kunde 1 had the lowest (1.7) as shown in Table 1. Under CaCl_2 , there were no significant differences at $p \leq 0.05$ in K80 and M66 for treatments 0 and 4 dS/m in all varieties as shown in Table 2.

Effect of NaCl and CaCl_2 levels on mean germination time

It was found that 0 dS/m resulted in a higher mean germination time while treatment 12 dS/m had the lowest. Under NaCl, Kunde 1 recorded the lowest mean germination time (0.2) while M66 had the highest (1.1) at 12 dS/m more also, significant differences in mean germination time were noted in all varieties between treatments 0 and 12 dS/m shown in Table 1. Under $\text{CaCl}_{2(\text{aq})}$, the mean germination time at treatments 8 and 12 dS/m were highly significant in all varieties as shown in Table 2.

Table 1: Effects of NaCl(aq) levels on germination parameters on four varieties of cowpea.

	Treatment	K80	M66	KUNDE 1	KVU 27-1
Days to emergence	0	6.0± 0.0 ^a	5.0±0.0	6.0±0.0	7.0±0.0
	4	7.7±0.3 ^b	6.0±0.0	9.0±0.0	7.0±0.0
	8	8.0±0.0 ^b	6.0±0.0	9.0±0.0	7.0±0.0
	12	8.0±0.0 ^b	7.0±0.0	10.0±0.0	8.0±0.0
LSD		0.4			
F value		33.00			
P value		0.0001			
Total seeds germinated.	0	10.0±0.0	9.7± 0.3 ^c	9.7±0.3 ^d	10.0±0.0 ^d
	4	8.0±0.0	10.0±0.0 ^c	7.3±0.3 ^c	9.0±0.0 ^c
	8	7.0±0.0	8.7±0.3 ^b	6.3±0.3 ^b	8.3± 0.3 ^b
	12	3.0±0.0	7.3±0.3 ^a	1.7±0.3 ^a	4.7± 0.3 ^a
LSD			0.71	0.78	0.55
F value			17.22	101.58	97.33
P value			0.001	0.0001	0.0001
Mean Germination Time	0	1.7±0.0 ^c	1.8±0.0 ^c	1.5±0.0 ^c	1.4±0.0 ^c
	4	0.9± 0.0 ^b	1.7±0.0 ^c	0.8±0.0 ^b	1.3±0.0 ^c
	8	0.8±0.0 ^b	1.5±0.1 ^b	0.7±0.0 ^b	1.0±0.1 ^b
	12	0.4±0.0 ^a	1.1±0.1 ^a	0.2±0.0 ^a	0.6±0.0 ^a
LSD		0.10	0.11	0.10	0.12
F value		323.02	47.67	258.0	45.70
P value		0.0001	0.0001	0.0001	0.0001
Mean Germination Rate	0	0.4±1.8 ^a	0.6±0.0 ^a	0.6±0.0 ^a	0.7±0.0 ^a
	4	1.0±0.0 ^b	0.6±0.0 ^a	1.2±0.0 ^b	0.8±0.0 ^a
	8	1.1± 0.0 ^b	0.7±0.0 ^a	1.4±0.0 ^c	0.8±0.0 ^a
	12	3.5±0.0 ^c	1.0±0.0 ^b	6.3±0.0 ^d	1.7±0.0 ^b
LSD		0.10	0.10	0.10	0.10
F value		1.64	32.25	5010.33	198.0
P value		0.255	0.0001	0.0001	0.0001
Final Germination Percentage.	0	99.3±0.3 ^d	97.2±0.4 ^d	96.7±0.2 ^d	99.0±0.6 ^d
	4	81.0±0.6 ^c	99.0±0.7 ^c	73.1±0.6 ^c	91.7±0.9 ^c
	8	71.0±0.6 ^b	87.2±0.4 ^b	64.1±0.5 ^b	82.8±1.5 ^b
	12	26.6±0.8 ^a	71.0±0.6 ^a	17.6±0.7 ^a	47.1±0.2 ^a
LSD		1.44	1.15	1.24	2.13
F value		2495.54	675.61	3896.87	638.280.
P value		0.0001	0.0001	0.0001	0.0001
Seedling Vigour	0	1617.7±2.5 ^d	1758.0±4.8 ^d	1677.4±0.8 ^d	1517.0±16.4 ^d
	4	1053.6±0.2 ^c	1717.7±0.6 ^c	831.5±1.4 ^c	1274.5±0.3 ^c
	8	852.1±4.7 ^b	1401.7±1.0 ^b	705.8±1.9 ^b	1124.4±0.3 ^b
	12	299.1±1.1 ^a	1064.7±2.4 ^a	182.6±1.3 ^a	614.9±0.2 ^a
LSD		6.26	5.91	3.20	19.09
F value		4.1	1.6	2.0	2171.6
P value		0.0001	0.0001	0.0001	0.0001

Mean values in same column denoted by similar letters are not significantly different at $p \leq 0.05$. F value at 5% significance level. Mean separated using LSD at 5% confidence interval.

Table 2: Effects of CaCl₂(aq) levels on germination parameters on four varieties of cowpea.

	Treatment	K80	M66	KUNDE 1	KVU 27-1
Days to emergence	0	6.0±0.0	5.0±0.0	6.0±0.0	7.0±0.0
	4	8.0±0.0	5.0±0.0	6.0±0.0	7.0±0.0
	8	8.0±0.0	6.0±0.0	6.0±0.0	7.0±0.0
	12	9.0±0.0	6.0±0.0	7.0±0.0	8.0±0.0
LSD					
F value					
P value					
Total seeds germinated.	0	9.7± 0.3 ^c	10.0± 0.0 ^b	9.7± 0.3 ^{bc}	10.0±0.0 ^d
	4	9.7± 0.3 ^c	10.0 ±0.0 ^b	9.3 ±0.3 ^b	9.3± 0.3 ^c
	8	6.3± 0.3 ^b	9.7±0.3 ^{ab}	9.0± 0.0 ^b	7.0± 0.0 ^b
	12	4.7± 0.3 ^a	9.0± 0.0 ^a	7.0± 0.0 ^a	6.0± 0.0 ^a
LSD		0.79	0.44	0.57	0.39
F value		56.25	8.00	25.83	129.0
P value		0.0001	0.009	0.0001	0.0001
Mean Germination Time	0	1.7± 0.0 ^d	1.9±0.0 ^c	1.6±0.0 ^c	1.4±0.0 ^c
	4	1.2±0.0 ^c	1.8±0.0 ^b	1.6±0.0 ^c	1.4±0.0 ^c
	8	0.7±0.9 ^b	1.8±0.0 ^b	1.4±0.1 ^b	1.0±0.1 ^b
	12	0.6±0.0 ^a	1.5±0.0 ^a	1.0±0.1 ^a	0.7±0.0 ^a
LSD		0.05	0.08	0.11	0.10
F value		441.03	40.25	36.33	64.44
P value		0.0001	0.0001	0.0001	0.0001
Mean Germination Rate	0	0.6 ±0.0 ^a	0.5±0.0 ^a	0.6±0.0 ^a	0.6±0.0 ^a
	4	0.8 ±0.0 ^b	0.5±0.0 ^a	0.6±0.0 ^a	0.7±0.0 ^a
	8	1.3±0.0 ^c	0.5±0.0 ^a	0.7±0.0 ^a	0.9±0.0 ^b
	12	1.8±0.0 ^d	0.7±0.0 ^b	1.0±0.0 ^b	1.3±0.0 ^c
LSD		0.10	0.10	0.10	0.10
F value		333.33	11.00	35.89	114.22
P value		0.0001	0.003	0.0001	0.0001
Final Germination Percentage.	0	99.0±0.7 ^c	99.0±0.6 ^b	96.2±0.6 ^c	99.7±0.3 ^c
	4	97.6±0.6 ^c	97.2±0.4 ^b	79.7±0.6 ^b	96.2±0.6 ^b
	8	62.0±1.2 ^b	98.3±0.9 ^b	89.0±0.6 ^c	73.7±1.9 ^a
	12	51.0±0.6 ^a	92.7±1.5 ^a	60.0±0.6 ^a	71.7±0.9 ^a
LSD		1.85	2.35	8.35	2.54
F value		955.86	9.66	20.87	182.77
P value		0.0001	0.005	0.0001	0.0001
Seedling Vigour	0	1564.1±0.4 ^d	1818.5±1.5 ^c	1663.4±1.7 ^d	1634.5±0.6 ^d
	4	1417.6 ±1.4 ^c	1758.3±2.4 ^b	1601.3±0.7 ^c	1563.2±0.6 ^c
	8	789.9±1.0 ^b	1819.1±1.4 ^c	1459.6±3.9 ^b	1063.0±0.7 ^b
	12	608.7±0.9 ^a	1457.7±2.1 ^a	910.0±0.8 ^a	991.3±0.5 ^a
LSD		2.31	4.45	5.05	1.25
F value		2.2	8220.5	2.5	3.0
P value		0.0001	0.0001	0.0001	0.0001

Mean values in same column denoted by similar letters are not significantly different at $p \leq 0.05$. F value at 5% significance level. Mean separated using LSD at 5% confidence interval.

Effect of NaCl and CaCl₂ levels on mean germination rate

As salinity levels increased, the mean germination rate increased among the four varieties. Under NaCl, Variety Kunde 1 had highest mean germination rate (6.3) compared to the other varieties at treatment 12 dS/m although Kunde 1 recorded a significant differences as salinity levels increased. Also, there was no significant differences at $p \leq 0.05$ in K80, M66 and KVVU 27-1 at treatments 4 and 8 dS/m as shown in Table 1. Under CaCl_{2(aq)}, there were significant differences in mean germination rate in K80 as levels increased as shown in Table 2.

Effect of NaCl and CaCl₂ levels on final germination percentage

As salinity levels increased, the final germination percentage decreased among the four varieties. Under NaCl, treatment 0 was significant different to the other treatments in all varieties. Variety Kunde 1 had the least final germination percentage (17.6) among the four varieties as shown in Table 1. Under CaCl₂, no significant difference among treatments 4, 8 and 12 dS/m in M66 at $p \leq 0.05$ between the three levels and final germination percentage although M66 recorded the highest final germination percentage (92.7) among the four varieties as shown in Table 2.

Effect of different NaCl and CaCl₂ levels on seedling vigour

Both NaCl and CaCl₂ recorded significantly different in seedling vigour as the levels of increased in all the four varieties as shown in Table 1 and 2. In addition, Variety M66 had the highest seedling vigour (1064.7) while Kunde 1 had the lowest (182.6) as shown in Table 1.

DISCUSSION

The results obtained showed that germination of the selected cowpea varieties (K80, M66, Kunde 1 and KVVU 27-1) significantly affected above 8 dS/m. The four varieties took longer

to emerge as salinity increased progressively from the control to EC of 12 dS/m. According to Achakzai *et al.* (2010), salinity affects germination by decreasing the osmotic potential to such a point which retard or prevent uptake of water that is necessary for mobilization of nutrient required for emergence hence a longer period for breakdown of seed dormancy causing a delayed germination. The number of seeds which germinated decreased significantly from the control to EC of 12 dS/m. The results also corresponded to Khan *et al.* (2011), that germination was directly related to the amount of water absorbed by the seeds therefore delay in germination occurs when the amount of water absorbed is reduced due to high salt uptake seeds which retards the emergence of some seeds.

The seedling vigour was lower in high salinity levels. The results corresponded to Patel *et al.* (2010), who reported that there is significant reduction in seed germination, seedling vigour and shoot dry weight with increasing salinity-induced stress in cowpea (*Vigna unguiculata*) cultivars. In addition, Iqbal *et al.* (2000) noted that an increase in salinity levels decreases germination parameters; seedling growth and vigour which is directly related to water uptake by the seeds and the toxic effects of Na⁺ at high salt concentrations which causes physical damage to roots. The reduction in seedling vigour could be due to the negative effect of salts on the rate of photosynthesis (Qados, 2011). Salinity stress suppresses meristem activity and cell elongation due to the changes in plant-water relationships hence reduction in seedling vigour (Essa, 2002). Salinity directly or indirectly inhibits cell division and enlargement resulting in reduction in shoot length (Kumar *et al.*, 2012). Unbalanced nutrition, reduced development and differentiation of tissues and disturbed avoidance mechanism as a result of salinity

stress is another reason that can be attributed to seedling vigour reduction (Ali *et al.*, 2004). The germination percentage, mean germination rate and time, and seedling vigour index studied for the four varieties were adversely affected by salinity level increased. In agreement with the findings of this study Munns *et al.* (2006) documented that the toxic effects of Na⁺ and Cl⁻ ions on germinating seeds affect germination percentage, germination rate, energy of germination and seedling vigour index. The observed decrease in the germination percentage may be as a result of decreased water uptake which could be attributed to a decrease in osmotic potential. Water is necessary for softening the seed coat increasing the seed permeability and mobilization of nutrients that enhances germination (Kaymakanova, 2009). The observed decrease in germination could also be as a result of toxicity of the salts ions to the developing embryo following germination which causes reduction of germination percentages and rates (Kaymakanova, 2009; Marambe & Ando, 1995).

The results of this study also correspond with Rahman *et al.* (2013) who reported a reduction in germination percentage in beans (*Phaseolus vulgaris*) that was directly proportional to the concentration of salt in the medium. Kumar *et al.* (2012) also observed reduced percentage of germination in wheat (*Triticum* sp) could be due to an increase in the soil osmotic pressure as a result of highly concentrated soil solution. Tolerance to salinity varies in different crop varieties. M66 was superior under high salinity levels because it had a vigorous emergence, highest seedling vigour and final germination percentage at 12 dS/m while Kunde 1 was the least tolerant cowpea variety based on slow emergence, lowest seedling vigour and final germination percentage at 12 dS/m.

Conclusion and Recommendations

From the results, it's clear that high salt levels inhibit the germination performance of cowpea. Sodium and calcium chloride levels above 8 dS/m caused a significant effect on germination of different cowpea varieties. The results provide evidence of differences in salinity tolerant of the commonly grown varieties in Kenya. M66 variety was the most tolerant while Kunde 1 is the least tolerant. Farmers in areas with higher salinity should adopt growing M66 cowpea.

Data Availability

The data that support the findings of this study are included within the article.

Disclosure

The authors declare that this is an original article which has not been published before and is not under consideration in another journal.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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